RESEARCH NOTE

Analysis of virulence factors in cases of enterococcal endocarditis

L. Baldassarri[1], R. Creti[1], C. R. Arciola[2], L. Montanaro[2], M. Venditti[3] and R. Di Rosa[3]

[1]Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Rome, [2]Istituti Ortopedici Rizzoli, Bologna and [3]Dipartimento di Medicina Clinica, Università 'La Sapienza', Rome, Italy

ABSTRACT

Eleven isolates of *Enterococcus faecalis* causing endocarditis were screened for possible virulence factors with PCR and phenotypic assays. The gene coding for the enterococcal surface protein (*esp*) was detected in one isolate only, and haemolysin was produced by two isolates. Aggregation substance, biofilm formation and gelatinase were present in seven, nine and eight isolates, respectively. Predisposing factors, particularly hospitalisation and multiple antibiotic therapy, appeared to be more relevant to the development of enterococcal endocarditis following bloodstream infections than the pattern of virulence factors.

Keywords Endocarditis, *Enterococcus faecalis*, virulence factors

Original Submission: 11 February 2004; **Revised Submission:** 19 April 2004; **Accepted:** 9 May 2004

Clin Microbiol Infect 2004; 10: 1006–1008
10.1111/j.1469-0691.2004.00999.x

Enterococci are recognised nosocomial pathogens, with *Enterococcus faecalis* and *Enterococcus faecium* being responsible for a large proportion of urinary tract and bloodstream infections in intensive care units. Endocarditis is a well-known complication of enterococcal bacteraemia that typically occurs on damaged (native valve endocarditis) or prosthetic (prosthetic valve endocarditis) heart valves. Infective endocarditis can lead to damage or destruction of heart valves, resulting, in the most severe cases, in heart failure and death. Possible enterococcal virulence factors involved in endocarditis have been reviewed previously [1], but none of those considered could account for the development of endocarditis. In a large collection of clinical and environmental isolates examined, isolates from urinary tract infections appeared to possess more virulence factors than those from other clinical or environmental sources [2]. In the present study, 11 cases of *E. faecalis* endocarditis were investigated. Possible predisposing factors and the patients' conditions were examined, and the causative isolates were characterised for putative virulence factors.

Cases of endocarditis were classified as 'definite endocarditis' according to the Duke criteria [3]. All patients had been hospitalised at the Policlinico of the University 'La Sapienza' in Rome, Italy, over a 4-year period. *E. faecalis* isolates from blood were identified and speciated according to standard methods, and verified by a species-specific 16S ribosomal PCR [4]. Production of gelatinase was determined on plates containing trypticase soy agar supplemented with skimmed milk 1.5% (w/v) [5]. Haemolysin production was evaluated on Columbia agar base supplemented with fresh human blood 5% v/v [2]. Biofilm formation was evaluated with a quantitative adherence assay as described previously [6]. The presence of the putative virulence factor genes for enterococcal surface protein (*esp*), aggregation substance (AS) (*asa1*), collagen-binding adhesin (*ace*) and endocarditis antigen (*efaA*) were investigated with PCR as described previously [2], with reference strains being used as positive controls [2].

The clinical features of the 11 cases of enterococcal endocarditis are listed in Table 1. There were four cases of native valve endocarditis and seven of prosthetic valve endocarditis. In all but two cases, predisposing factors were present, of which hospitalisation and multiple antibiotic therapy were the most frequent. One isolate was vancomycin-resistant, while three exhibited high-level resistance to aminoglycosides (Table 1).

One isolate was *esp*-positive, seven isolates were *asa1*-positive, and *ace* and *efaA* were present in all isolates (Table 2). Haemolysin and gelatinase were produced by two and eight isolates, respectively. Biofilm production was demonstrated with nine isolates. A similar analysis (data not

Corresponding author and reprint requests: L. Baldassarri, Laboratorio di Malattie Batteriche Respiratorie e Sistematiche, Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Viale Regina Elena 299, 00161 Rome, Italy
E-mail: baldassa@iss.it

Table 1. Microbiological and clinical features of 11 cases of *Enterococcus faecalis* endocarditis

Strain	Sex/Age	Source of sepsis/predisposing factors	Underlying heart disease	Site of infection	Acquired antibiotic resistance	Therapy	Outcome
EE1	M/84	TURP, hosp, mat	DVD	Mitral valve	HLRA	AMP + CRO, then TEC	Cured [13]
EE2	F/76	hosp	RVD	Bioprosthetic mitral valve	–	AMP + GEN	Cured
EE3	M/71	Urethral stenosis with endoscopic procedures, hosp, mat	DVD	Aortic valve	–	AMP + GEN	Cured
EE4	M/38	None	Unknown	Mitral valve	HLRA	AMP + CRO	Cured[a]
EE5	M/68	Diverticular disease with endoscopic procedures, hosp, mat	RVD	Bioprosthetic mitral valve	VanA	AMP + GEN	Cured [14]
EE6	M/48	hosp	RVD	Prosthetic mitral and aortic valve	HLRA	AMP + GEN	Died[b]
EE7	M/28	IVDU	Unknown	Mitral valve	–	TEC + GEN	Cured
EE8	M/67	Hip arthroplasty, hosp, mat	DVD	Prosthetic aortic valve	–	AMP + GEN	Cured
EE9	M/69	hosp, mat	DVD	Prosthetic aortic valve	HLRA	AMP + CRO	Cured
EE10	M/40	None	RVD	Prosthetic aortic valve	–	AMP + GEN	Cured
EE11	F/65	hosp, mat	DVD	Intraventricular patch	–	AMP + GEN	Cured

TURP, trans-urethral prostatectomy; DVD, degenerative valvular disease; RVD, rheumatic valvular disease; hosp, hospitalisation in the previous 6 months; mat, multiple antibiotic therapy; IVDU, intravenous drug user; HLRA, high-level resistance to aminoglycosides (streptomycin and gentamicin); AMP, ampicillin; CRO, ceftriaxone; GEN, gentamicin; TEC, teicoplanin.
[a]Surgical replacement of dysfunctional valve after the end of treatment: no bacterial growth at culture.
[b]On the third day of empirical antibiotic treatment, the patient underwent emergency surgical replacement of prosthetic valve and died of cardiogenic shock 2 days after surgery; culture of the aortic valve yielded *E. faecalis* growth.

shown) conducted on 20 bacteraemia isolates during the same period in this hospital showed similar frequencies of the examined factors, with two exceptions: *esp* was detected in 50% of cases, and all isolates demonstrated biofilm formation.

Of particular interest were two isolates characterised in this study (EE3 and EE4) that lacked all of the putative virulence factors tested except gelatinase. EE4 caused massive destruction of the cardiac valves in a man aged 38 years with no previous history of significant heart disease. The apparent lack of predisposing conditions would suggest that EE4 was a particularly aggressive microorganism, and the valve destruction was consistent with the ability of this isolate to produce a protease, which appeared to be the only possible virulence factor. As for EE3, at least one of the urinary tract infections documented for this patient was caused by *E. faecalis*, so it may be hypothesised that translocation occurred. Unfortunately, this could not be confirmed, as the urine isolate was not stored.

Table 2. Putative virulence factors of endocarditis-causing *Enterococcus faecalis*

Strains	Biofilm	Haemolysin	*asa1*	*esp*	Gelatinase	*ace*	*EfaA*
EE1	+	–	+	–	+	+	+
EE2	+	–	–	–	+	+	+
EE3	–	–	–	–	+	+	+
EE4	–	–	–	–	+	+	+
EE5	+	–	+	–	–	+	+
EE6	+	–	+	–	–	+	+
EE7	+	–	+	–	–	+	+
EE8	+	–	+	–	+	+	+
EE9	+	+	+	–	+	+	+
EE10	+	–	–	–	+	+	+
EE11	+	+	+	+	+	+	+

asa1, aggregation substance; *esp*, enterococcal surface protein; *ace*, collagen-binding adhesin; *EfaA*, endocarditis antigen.

Of all the putative virulence factors described in the literature, AS and extracellular polysaccharides were detected in most of the isolates; no difference was observed in the distribution of virulence factors among endocarditis or bacteraemia isolates, except for *esp*, which was more frequent in the latter group.

Evidence for the role of gelatinase in endocarditis comes from a rabbit model of catheter-induced endocarditis, where gelatinase-producing isolates were associated with a more rapidly fatal disease [7]. Involvement of AS in virulence has been suggested by several in-vitro and animal studies (reviewed in McCormick *et al*. [1]); in particular, Chow *et al*. [5] concluded that AS contributed to an increase in the size of valve vegetation.

A role for biofilm formation in the pathogenesis of enterococcal infections has also been suggested [6], with production linked to stress conditions and survival inside macrophages [6,8,9]. Biofilm production is known to be important in the establishment of infections by opportunistic coagulase-negative staphylococci and *Staphylococcus aureus*, particularly those infections involving prosthetic materials.

As in previous studies involving clinical and environmental isolates [2,10], all the isolates were *ace*- and *efaA*-positive, although expression of *ace* or *efaA* may be modulated *in vivo*, thus contributing to the pathogenesis of endocarditis. In addition, isolates negative for haemolysin and gelatinase production might still harbour the respective genes.

In conclusion, the specific pattern of the virulence factors tested does not appear to correlate with the ability of *E. faecalis* to cause endocarditis. Indeed, endocarditis isolates seem to have fewer virulence factors compared to isolates from other sources. However, the ability to express specific traits, such as gelatinase, might correlate with the ability of strains to cause the disease. Predisposing factors for the development of endocarditis appeared to be sex (males were more frequently affected), age and hospitalisation, which confirmed previous findings [11,12], as well as underlying heart disease and enterococcal bacteraemia.

ACKOWLEDGEMENTS

This work was partially supported by a grant from the Italian Ministry of Health, Project 1% 'Characterisation of the genomic bases of viral, bacterial, and parasitic infections for control strategies' (0AD/F) to LB.

REFERENCES

1. McCormick JK, Hirt H, Dunny GM, Schlievert P. Pathogenic mechanisms of enterococcal endocarditis. *Curr Infect Dis Rep* 2000; **2**: 315–321.
2. Creti R, Imperi M, Bertuccini L, Orefici G, Di Rosa R, Baldassarri L. A survey for virulence determinants among *E. faecalis* isolated from different sources. *J Med Microbiol* 2004; **53**: 13–20.
3. Durack DT, Lukes AS, Bright DK, the DUKE Endocarditis Service. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. *Am J Med* 1994; **96**: 200–209.
4. Dicuonzo G, Gherardi G, Lorino G *et al.* Antibiotic resistance and genotypic characterization by PFGE of clinical and environmental isolates of enterococci. *FEMS Microbiol Lett* 2001; **201**: 205–211.
5. Chow JW, Thal LA, Perry MB *et al.* Plasmid associated hemolysin and aggregation substance production contribute to virulence in experimental enterococcal endocarditis. *Antimicrob Agents Chemother* 1993; **37**: 2474–2477.
6. Baldassarri L, Cecchini R, Bertuccini L *et al. Enterococcus* spp. produces slime and survives in rat peritoneal macrophages. *Med Microbiol Immunol* 2001; **190**: 113–120.
7. Gutschik E, Moller S, Christensen N. Experimental endocarditis in rabbits. Significance of the proteolytic capacity of the infecting strains of *Streptococcus faecalis*. *Acta Pathol Microbiol Scand (B)* 1979; **87**: 353–362.
8. Baldassarri L, Bertuccini L, Ammendolia MG *et al.* Receptor-mediated endocytosis (rme) of biofilm-forming *Enterococcus faecalis* by rat peritoneal macrophages. *Ind J Med Res* 2004; **119**: 129–133.
9. Hufnagel M, Koch S, Creti R, Baldassarri L, Huebner J. A putative sugar-binding transcriptional repressor in a novel gene locus in *Enterococcus faecalis* contributes to biofilm formation and prolonged bacteremia in mice. *J Infect Dis* 2004; **189**: 420–430.
10. Duh RW, Singh KV, Malathum K, Murray BE. In vitro activity of 19 antimicrobial agents against enterococci from healthy subjects and hospitalised patients and use of an *ace* gene probe from *Enterococcus faecalis* for species. *Microb Drug Resist* 2001; **7**: 39–46.
11. Fernandez-Guerrero ML, Herrero L, Bellver M, Gadea I, Roblas RF, De Gòrgolas M. Nosocomial enterococcal endocarditis: a serious hazard for hospitalized patients with enterococcal bacteraemia. *J Intern Med* 2002; **252**: 510–515.
12. Barrau K, Boulamery A, Imbert G *et al.* Causative organisms of infective endocarditis according to host status. *Clin Microbiol Infect* 2004; **10**: 302–308.
13. Tarasi A, Capone A, Venditti M. Options for the treatment of patients with severe infections caused by enterococci exhibiting high level resistance to aminoglycoside. *J Antimicrob Chemother* 1998; **42**: 113–114.
14. Carfagna P, Tarasi A, Cassone M, Del Grosso MF, Bianco G, Venditti M. Prosthetic biologic valve endocarditis caused by a vancomycin resistant (VanA) *Enterococcus faecalis*: case report. *J Chemother* 2000; **12**: 416–420.